Mycobacterium abscessus Displays Fitness for Fomite Transmission

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ABSTRACT *Mycobacterium abscessus* is a rapidly growing nontuberculous mycobacterium (NTM) increasingly reported in soft tissue infections and chronic lung diseases, including cystic fibrosis. The environmental source of *M. abscessus* has not been definitively identified, but NTM have been detected in soil and water. To determine the potential of soil-derived *M. abscessus* as an infectious source, we explored the association, growth, and survival of *M. abscessus* with defined mineral particulates, including kaolin, halloysite, and silicone dioxide, and house dust as possible *M. abscessus* fomites. *M. abscessus* physically associated with particulates, and the growth of *M. abscessus* was enhanced in the presence of both kaolin and house dust. *M. abscessus* survived desiccation for 2 weeks but was not viable after 3 weeks. The rate of decline of *M. abscessus* viability during desiccation was reduced in the presence of house dust. The evidence for enhanced growth and survival of *M. abscessus* during alternating growth and drying periods suggests that dissemination could occur when in wet or dry environments. These studies are important to understand environmental survival and acquisition of NTM.

IMPORTANCE The environmental source of pulmonary *Mycobacterium abscessus* infections is not known. Fomites are nonliving carriers of infectious agents and may contribute to acquisition of *M. abscessus*. This study provides evidence that *M. abscessus* growth is enhanced in the presence of particulates, using kaolin, an abundant natural clay mineral, and house dust as experimental fomites. Moreover, *M. abscessus* survived desiccation for up to 2 weeks in the presence of house dust, kaolin, and several chemically defined mineral particulates; mycobacterial viability during extended periods of desiccation was enhanced by the presence of house dust. The growth characteristics of *M. abscessus* with particulates suggest that a fomite mechanism of transmission may contribute to *M. abscessus* acquisition, which may lead to strategies to better control infections by *M. abscessus* and related organisms.

KEYWORDS acquisition, cystic fibrosis, desiccation, microbe-mineral interactions, nontuberculous mycobacteria

Infections attributable to nontuberculous mycobacteria (NTM) are especially troubling to patients, clinicians, and public health professionals due to the notable intrinsic antibiotic resistance and poor rates of eradication of these organisms (1). The incidence of NTM infections in patients with cystic fibrosis (CF) is more than 1,000 times that in healthy individuals (2), and the appearance of persistent NTM cultures in CF patients is associated with worse outcomes and lung disease progression (3–6). The *Mycobacterium abscessus* group (MABSC) comprises rapidly growing NTM with increasing prevalence in chronic lung infections (3, 4, 7). The MABSC contains three subspecies, namely, *M. abscessus* subsp. *abscessus* (*M. abscessus*), *M. abscessus* subsp. *massiliense*, and

Received 6 June 2017 Accepted 20 July 2017
Accepted manuscript posted online 28 July 2017

Citation Malcolm KC, Caceres SM, Honda JR, Davidson RM, Epperson LE, Strong M, Chan ED, Nick JA. 2017. *Mycobacterium abscessus* displays fitness for fomite transmission. Appl Environ Microbiol 83:e00562-17. <https://doi.org/10.1128/AEM.00562-17>.

Editor Andrew J. McBain, University of Manchester

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FIG 1 Physical association of *M. abscessus* with defined and environmental particulates. (A to C) Light microscopy of *M. abscessus* grown in the absence (A) or presence of kaolin (B) or house dust 1 (C) for 7 days and adhered to slides and Ziehl-Neelsen stained. *M. abscessus* (stained pink and highlighted by black arrows) is distinguished from particulate matter (purple [open arrows]). Clumping of mycobacteria is typical after longer culture times, as evident in panel A. Black scale bars, 10 μm . (D to F) *M. abscessus* grown for 1 day in the absence (D) or presence of kaolin (E) or SiO_2 (F) for 24 h was stained with Syto 9 (green) and propidium iodide (red); fluorescence was overlaid on photomicrographs. White scale bars, 50 μm .

M. abscessus subsp. *bolletii*, which are difficult to differentiate with common microbiological techniques but are distinguished by differences in therapeutic responses (8). Recent evidence for patient-to-patient transmission of MABSC has raised questions concerning the potential mechanism(s) by which the bacteria could be acquired (9, 10).

Evidence for the generation and survival of MABSC in airborne droplets suggests transmission through this route (10, 11). However, MABSC strains implicated in person-to-person transmission, notably of *M. abscessus* subsp. *massiliense*, represent only a fraction of strains found in infected patients (10), suggesting that an environmental source of MABSC infection is important, but its exact nature is not known. NTM are found in soil and water environments (12–16), but most of the isolated pathogenic organisms are within the *M. avium* complex, and MABSC members are rarely detected (17–20). Individual *M. avium* complex members may have distinct environmental niches (13, 14), suggesting a complex interplay between environment and host acquisition.

Organisms within the MABSC are extremely hydrophobic with a predilection toward surface attachment and biofilm formation (21–23). Viable bacteria have previously been detected in house and environmental dust samples (24–26). We hypothesize that MABSC can be transmitted when attached to particulate fomites. As a proof of principle for fomite transmission, we present an *in vitro* model for the growth and survival of *M. abscessus* subsp. *abscessus* (*M. abscessus*), the most common infectious MABSC member, in the presence of chemically defined particles and house dust. The results of this study increase the understanding of potential survival capabilities of MABSC and suggest that acquisition could include an airborne environmental route mediated by fomites.

RESULTS

Physical association and enhanced growth of *M. abscessus* with particles. The airborne acquisition of MABSC requires a means to transmit the pathogen. Airborne transmission by droplets has been proposed, but we hypothesized that the direct association of *M. abscessus* with dust-like particles could provide a vehicle for acquisition. *M. abscessus* was grown in the presence of chemically defined particulates and house dust to better understand their interaction. We observed direct contact of *M. abscessus* with the widely found mineral clays kaolin (27) and halloysite (not shown), silicone dioxide, and sterile house dust (Fig. 1). By contrast, *M. abscessus* cultured in the absence of particulates grew as loosely associated aggregates or individual cells (Fig. 1A and D). Autofluorescence of house dust precluded an examination of these samples by fluorescence microscopy.

FIG 2 Particulates promote the growth and survival of *M. abscessus*. (A and B) Growth curves of the ATCC strain (A) and CF clinical strain CF016 (B) alone (black) or in the presence of kaolin (blue) or house dust sample 1 (red) over 7 days; the graphs on the right indicate the area under the curve (AUC) measurements for each curve. *, $P < 0.05$; **, $P < 0.01$ versus no dust by paired t test from 3 independent experiments. (C) Survival of stationary-phase *M. abscessus* grown in medium alone (black) or in the presence of kaolin (blue) or house dust sample 1 (red) was enumerated at the indicated days. *, $P < 0.05$; **, $P < 0.01$ for CFU/ml between medium and dust sample 1 by unpaired t test; $n = 3$ to 6 independent experiments.

To determine if the presence of particles provides a growth advantage, we measured bacterial growth over 7 days in the presence and absence of kaolin and house dust. We focused on kaolin for its chemically defined nature and natural occurrence and on house dust for its potential for indoor environmental exposure. Increases in the bacterial numbers of both the *M. abscessus* type strain ATCC 19977 and a CF isolate, CF0016, were observed when either kaolin or house dust was included compared with growth in medium alone (Fig. 2A and B). *M. abscessus* grown in the presence of kaolin and house dust achieved a higher density and the growth was significantly greater than that in medium alone as determined by area under the curve measurements and unpaired t tests (Fig. 2A and B, right panels). When stationary-phase *M. abscessus* was grown in the presence of particulates, the viability was not affected during early culture times; however, house dust prolonged survival compared with that of *M. abscessus* grown in medium or in the presence of kaolin after 6 days of culture (Fig. 2C).

***M. abscessus* survives desiccating conditions.** One potential mechanism of *M. abscessus* acquisition is by the inhalation of dry airborne dust particles carrying *M.*

FIG 3 *M. abscessus* survives desiccation. (A) Depiction of the experimental design. *M. abscessus* was grown in medium alone or in the presence of kaolin for 1 day. Cultures were centrifuged and pellets allowed to dry for 5 days. Dried pellets were resuspended in medium, and after growth for 24 h, bacteria were enumerated. (B) *M. abscessus* viability was assessed after 5 days of desiccation (red). Inoculum (open bars) and bacterial numbers at the start of drying (black) are also shown. The graph represents the means $\pm$ SEM from 5 experiments.

abscessus. To model this potential fomite acquisition, *M. abscessus* was grown in medium alone or in the presence of kaolin and was dried and reconstituted to determine bacterial survival (Fig. 3A). Viable *M. abscessus* was recovered from cultures dried for 5 days, but survival did not depend on kaolin (Fig. 3B). Furthermore, halloysite (a similar Al- and Si-containing mineral clay), silicone dioxide, or AlCl_3 did not alter *M. abscessus* survival (not shown) after drying.

We next measured the recovery of viable *M. abscessus* from dried cultures over longer times. A loss of viability was observed as early as 7 days after desiccation and was time dependent (Fig. 4). After 21 days of drying, no viable bacteria were recovered under any condition tested (limit of detection, 100 CFU/ml). Cultures of the ATCC type strain dried in the presence of three different house dust samples were somewhat protected from desiccation compared with *M. abscessus* alone or that dried in the presence of kaolin. The half-life of survival for *M. abscessus* grown in the presence of kaolin was not different from that of *M. abscessus* grown in medium alone (0.38 and 0.53 days, respectively) (Table 1). The half-life of *M. abscessus* survival after drying was increased significantly to more than 1 day in house dust samples (Table 1). Similar survival results were observed for two clinical strains of *M. abscessus* (Fig. 4B and C; Table 1).

DISCUSSION

MABSC acquisition is poorly understood. We have presented evidence that *M. abscessus* has an affinity to both defined and complex particulates and that in the presence of these particulates, *M. abscessus* displayed enhanced growth and fitness under desiccation conditions. These findings suggest that a mechanism of transmission could include environmental growth of *M. abscessus* in the presence of particulates, drying in a native environment, and dispersal of bacterium-associated particles. While our data suggest that *M. abscessus* displays fitness under desiccation conditions in the absence of particulates, its growth in a natural environment likely includes particulates, and we demonstrated an association of *M. abscessus* with particulates. Furthermore, house dust supported enhanced growth and survival of *M. abscessus*, suggesting that environmental particulates include additional nutrients or factors. A similar enhancing

FIG 4 *M. abscessus* survives desiccation for extended periods. (A) Depiction of the experimental design. *M. abscessus* was grown for 3 days (light gray) in medium alone or in the presence of particulates, dried for the indicated times, and regrown for 1 day (dark gray) and assessed for surviving bacteria. (B and C) Assessment of *M. abscessus* viability after drying of the ATCC type strain (B) and a CF clinical isolate, CF0016 (C), over 21 days in medium alone (black) or with kaolin (blue) or dust samples (red, open yellow, and orange). The small light brown circle represents the inoculum at day -3.

effect of indoor dust was observed on the growth of Gram-negative organisms under nutrient-poor conditions (28). Soluble factors are less likely to be involved, as the experimental design includes a washing step that reduces exposure to soluble material.

It is not known how *M. abscessus* survives desiccation, but it could involve several mechanisms. The retention of water by the waxy coating may provide an intrinsic advantage during times of desiccation. Furthermore, *M. abscessus* may adapt to dry environments by changing the expression of genes for channels, pores, or additional physical barriers. A mutational analysis of candidate genes may improve our understanding of desiccation survival. Finally, clumping or adherence to solids, as seen in Fig. 1, may provide additional measures to protect from desiccation. Similarly, the mechanisms of interaction of *M. abscessus* with the particles tested are not known. It is possible that specific physical properties of the studied dust particles, such as charge or structure, are important. In addition, *M. abscessus* surface molecules involved in the interaction are likely critical and could include proteins, lipids, and carbohydrates. The nature of these interactions requires further study.

The survival of other bacteria under desiccating conditions has been studied. *Pseudomonas aeruginosa* has poor fitness under desiccation conditions over 5 h, while *Staphylococcus aureus* is fully viable after this time (29). Poor *P. aeruginosa* survival was reported after >100 days on filter discs (30) and after 24 h when dried on glass coverslips (31, 32). Likewise, *Burkholderia* viability decreased 2 to 5 logs after 7 h of drying (33). Viable methicillin-resistant *S. aureus* was detected on glass and tile for more than 5 weeks in dried serum and was detected on stethoscopes (34). Nonpathogenic

TABLE 1 Half-life of *M. abscessus* viability after desiccation

Sample	Condition	Mean $t_{1/2}$ (days [SEM]) ^a	P value ^b
ATCC type strain	No dust	0.38 (0.003)	NA
	Kaolin	0.53 (0.13)	0.395
	HD ^c 1	1.41 (0.13)	0.015
	HD 2	1.30 (0.21)	0.049
	HD 3	1.23 (0.10)	0.013
	Composite HD	1.32 (0.08)	0.000087
CF0016	No dust	0.38 (0.01)	NA
	Kaolin	0.38 (0.01)	0.632
	HD 1	1.11 (0.03)	0.003
	HD 2	0.55 (0.08)	0.214
	HD 3	0.90 (0.26)	0.194
	Composite HD	0.85 (0.11)	0.046
CF0026	No dust	0.38 (0.003)	NA
	Kaolin	0.37 (0.004)	0.801
	HD 1	1.21 (0.14)	0.029
	HD 2	0.97 (0.30)	0.186
	HD 3	1.24 (0.23)	0.063
	Composite HD	1.14 (0.12)	0.006

^aValues of loss of *M. abscessus* viability in response to increasing times of desiccation represent the results from three independent experiments. Data were extracted from Fig. 4.

^bResults from *t* test versus no dust. NA, not applicable; boldface font indicates significance level of *P* < 0.05.

^cHD, house dust sample.

Methylobacterium species recovered from fan blades displayed desiccation resistance over 24 h (35). Importantly, these examples measured desiccation survival using nonnatural supports. By contrast, we detected viable *M. abscessus* up to 14 days after drying using chemically defined, natural, dust-like minerals and house dust samples. Our method also has an increased sensitivity, as bacteria grew overnight before enumeration. It is also evident that considerable loss of viability occurs during drying. The extensive waxy coat of mycobacteria may be important for survival under these conditions, potentially by retaining water. Most isolates of NTM and *M. tuberculosis* survived lyophilization for several years (36), and *M. intracellulare* survived drying on glass with a half-life of 2.3 days (37), a rate slightly higher than that seen in our experiments.

The occurrence of MABSC-associated particles in the environment has not been demonstrated. The genus *Mycobacterium* could be detected at low levels from environmental samples using 16S sequencing (38) (data not shown), but an identification at the species level is technically difficult. Future experiments will focus on identifying airborne MABSC. Importantly, few if any reports detected MABSC from environmental sources, including water and soil (17–20). It is possible that MABSC is exceedingly rare in the environment or that available detection techniques lack sensitivity. Of interest is the appearance of *M. abscessus* infections following trauma associated with the 2004 Thailand tsunami and with war wounds (39–42), suggesting that abrasions and wounds were directly infected from environmental sources. Nigerian diagnoses of NTM, including *M. abscessus*, coincided with the Harmattan, a period of increased wind laden with Saharan dust (43), and *Actinobacteria*, the phylum to which mycobacteria belong, were identified in Asian dust particles (25). However, no MABSC were detected in Finnish indoor house dust samples by PCR (24), which may lack sensitivity. In addition, environmental particles include Al- and Si-containing minerals (25, 44, 45), and these have proinflammatory activity that could promote a permissive environment for bacterial growth (44, 46). Interestingly, the distribution of kaolin overlaps with a high incidence of NTM in the southeastern United States (7, 27), but NTM incidence is also associated with humid environments (47). Although indoor environmental samples were used in this study, future experiments will explore the ability of outdoor samples, such as potting, peat, and forest soils (12, 48, 49), to support *M. abscessus* fitness.

In summary, we have demonstrated the fitness of *M. abscessus* to survive desiccating

conditions and to physically associate with particulates. Although *M. abscessus* appears to have intrinsic desiccation-induced fitness, the association with mineral particulates and house dust further promotes growth and survival fitness. These studies point to the possibility of a particulate-mediated route of infection by *M. abscessus* and will help us understand the control and acquisition of MABSC.

MATERIALS AND METHODS

Bacterial strains and culture. *M. abscessus* subsp. *abscessus* ATCC strain 19977^T/CIP104356^T was propagated from frozen aliquots in 7H9 broth supplemented with 10% albumin-dextrose-catalase (ADC), 2% glycerol, and 0.05% Tween 80 at 33°C with shaking at 300 rpm for 3 to 5 days. The smooth morphotype was used. Cultures were adjusted to an optical density at 600 nm (OD₆₀₀) of 1.0 in 0.9% NaCl, corresponding to approximately 1×10^8 CFU/ml. The clinical isolates, CF0016 and CF0026, were cultured as described above; these strains are smooth morphotypes obtained from the Colorado Cystic Fibrosis Research and Development Program Culture, Biorepository, and Coordinating Core at National Jewish Health. Large aggregates of *M. abscessus* were allowed to settle, and only washed cultures containing single cells or small clumps were used.

Particulate preparation. Kaolin (K1512), halloysite (685445), and silicone dioxide (S5631) were purchased from Sigma. House dust was prepared from vacuum cleaner bag contents that were filtered through a 100- μ m mesh and autoclaved. House dust 1 was derived from National Jewish Health conference rooms and offices, and house dusts 2 and 3 were from residential settings. All preparations were suspended to 1 mg/ml in sterile 0.9% saline before use. Each preparation was confirmed as sterile prior to use.

Particulate-associated growth. Particulate suspensions (100 μ l) or saline aliquots in 1.6-ml microcentrifuge tubes were inoculated with bacteria at 10^5 to 10^6 CFU/ml and incubated at room temperature for 10 to 20 min to allow time for bacteria to associate. Suspensions were centrifuged at $12,000 \times g$ for 5 min at 22°C, and the pellets were resuspended in 100 μ l RPMI medium containing L-glutamine, 10 mM HEPES, pH 7.4, and 2% pooled heat-inactivated platelet-poor human plasma (complete RPMI medium). The washing procedure removes soluble components from the sample; mammalian culture medium was used to reduce the optimal growth of bacteria, to better mimic physiological conditions, and to ensure consistency with future experiments involving immune cells. Cultures were incubated for up to 10 days at 37°C with rocking at 50 oscillations per minute. The initial dilution was performed by adding 900 μ l 0.1% Triton X-100 followed by vigorous vortex mixing for 20 s. Serial dilutions in saline were used for plating on 7H10 agar supplemented with 10% oleic acid-albumin-dextrose-catalase, and CFU were measured after 3 to 5 days of incubation at 37°C. In experiments testing the bacterial survival after drying, cultures were grown in the presence or absence of particulates for 1 or 3 days and centrifuged at $12,000 \times g$ for 5 min, and the medium was removed. Pellets were air dried and subsequently capped and stored at room temperature for times indicated in Fig. 3 and 4. Surviving bacteria were revived by adding fresh complete RPMI medium and regrown at 37°C for 24 h followed by CFU enumeration as described above.

Microscopy. *M. abscessus* grown in the absence or presence of kaolin for 7 days was spotted on glass slides, air dried, heat fixed, and stained using the TB carbofuchsin ZN kit (BD) according to the manufacturer's protocol. Briefly, carbofuchsin was heated on the slides, rinsed with water, decolorized with 3% acid alcohol, and counterstained with methylene blue. For fluorescence detection, bacteria were grown for 24 h in the absence or presence of particulates. Portions of the cultures were diluted 1:10 in a solution containing 500 nM Syto 9 and 30 μ M propidium iodide and were incubated for 10 min at room temperature in the dark and then in 3.7% formaldehyde for an additional 10 min. Particles and bacteria were pelleted by centrifugation at $4,000 \times g$ for 5 min at 22°C and resuspended in water. Portions of the suspensions were spotted on microscope slides and air dried. Slides were visualized by confocal fluorescence microscopy.

Statistical analysis. Data were analyzed by *t* tests comparing growth in the presence or absence of particulates. All data were normally distributed. Area under the curve measurements were determined using GraphPad Prism (version 6.02).

ACKNOWLEDGMENTS

This research was funded in part by grants from the Cystic Fibrosis Foundation to K.C.M. (MALCOL14G0) and J.A.N. (NICK14G0) and was supported by resources provided by the Colorado Cystic Fibrosis Research and Development Program.

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